REMARKS

Entry of the foregoing and reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested in light of the remarks which follow.

Claims 1-13 are currently pending. Claims 3, 4 and 6-13 stand withdrawn. Claims 1 and 2 are amended herein. New claims 14-16 are added herein. The title of the present invention is amended. Basis for the amendments to claim 1 may be found at least on page 6, lines 17 and 26-29. Basis for the amendments to claim 2 may be found at least on pages 12 to 18 of the specification. Basis for new claims 14-16 may be found in claims 1 and 2 as-filed. Thus, no prohibited new matter is added herein.

Specification

As suggested by the Examiner, the title of the present application has been amended. The title now recites "Enumeration of CD4+ Lymphocytes".

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 2 and 5 stand rejected under 35 U.S.C. § 112, second paragraph, as purportedly indefinite.

Applicants have amended the claims to recite CD4+ (CD4 positive) cells, to clarify them as opposed to a reference population. The claims are further amended to provide antecedent basis for the steps reciting "CD4+ lymphocytes". The preamble of claim 1 has been amended in this regard as well, to correspond with the main body of the claim.

Independent claim 1 stands rejected as it is purportedly unclear as to how steps (a) and (c) of claim 1 differ. Applicants note that step (a) requires only that the total white blood cell population be identified, whereas step (c) requires that the number of white blood cells per volume be determined. In other words, step (a) is not dependent on the volume of blood, but is rather an identification step for purposes of using the identified white blood cell population as a reference against which the percentage of CD4+ lymphocytes can be calculated. A more detailed explanation of these steps is set out on page 13, lines 1-5 and page 17, lines 1-6

(step 1(a)); and on page 12, lines 4-8 and page 17, lines 10-14 (step 1(c)). Furthermore, claim 1 is amended herein to address this issue.

In light of the above, Applicants request that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

Rejections Under 35 U.S.C. § 102

Claims 1 and 2 stand rejected under 35 U.S.C. § 102 as purportedly anticipated by Melnicoff (U.S. Patent No. 5,385,822) ("Melnicoff") in light of Dorland's Illustrated Medical Dictionary, 2005 ("Dorland"). Applicants respectfully traverse.

To anticipate a claimed invention under §102, a reference must teach each and every element of the claimed invention. *See Lindeman Machinenfabrik GmbH v. American Hoist and Derrick Company*, 221 USPQ 481, 485 (Fed. Cir. 1984). Applicants submit that Melnicoff fails to recite each element of the present invention.

Independent claim 1, as recited herein, is directed to a method of enumerating the number of CD4+ lymphocytes in a cell sample, comprising identifying the total white blood cell population as a reference population from which the CD4+ lymphocytes are subsequently measured; determining the percentage of CD4+ lymphocytes as a function of the total white blood cell reference population; determining the number of white blood cells per volume of blood; and calculating the absolute number of CD4+ lymphocytes in the sample by multiplying the percentage of CD4+ lymphocytes, by the white cell count. Applicant respectfully submits that Melnicoff does not disclose a method of enumerating the number of CD4+ lymphocytes in a blood cell sample by identifying the total leukocyte population as the primary anchor reference for the enumeration of the lymphocytes. (Col. 19, ln 15- col. 21, ln 50 EX 3a steps 7-8 and 16- 17, step 11).

Applicants note that the term "leukocyte count", as used in Melnicoff, is a descriptive error. Applicants submit that it would have been clear to the skilled artisan at the time Melnicoff was filed, that "leukocyte count" refers to the use of a total lymphocyte count (and not a leukocyte count) for use in determining lymphocyte count. It appears that this error is the result of an incorrect descriptive term used in the drafting of the Melnocoff application. From review of the context in which "leukocyte count" is used in Melnicoff, it appears that the error was not due to due to

the belief that the total leukocyte count was being identified. Therefore, Melnicoff is not an enabling disclosure for the use of the total leukocyte population, as a means of enumerating the number of CD4+ lymphocytes in a blood sample.

A review of Melnicoff makes it clear that a total lymphocyte count was the intended purpose of the methods disclosed therein. At col. 6, ln 8-26, Melnicoff refers to use of a total lymphocyte count (TLC) in the description of how an absolute CD4+ lymphocyte count is derived by explaining that a TLC is required, in the context of generation of a dual platform absolute CD4+ lymphocyte count, using flow cytometry. Melnicoff explains that the TLC is obtained by multiplying the white blood cell count (WCC, or leukocyte count as per Dorland's Illustrated Medical Dictionary) by the white blood cell differential lymphocyte percentage (lymphocyte% of total white blood cells) in order to obtain a total lymphocyte count (TLC) (*see* col. 6, ln 10-18). The TLC is then multiplied by the % CD4 lymphocytes derived by flow cytometry to obtain a CD4+ lymphocyte count per liter blood" (*see* col. 6, ln 13-15).

Melnicoff cites the use of a TLC in the context of dual platform CD4+ lymphocyte enumeration as problematic (in view of the variability introduced into CD4+ lymphocyte counting) when TLC is used in the conventional TLC based dual platform system. This was particularly relevant to Melnicoff, which does not rely on use of the TLC and uses an independent system. The independent method described by Melnicoff specifically defines T-cells by direct use of CD2 or CD3 with enzymatically generated fluorescence (and does not use a TLC from a haematology analyser). Therefore, the methods of Melnicoff would not have been affected by the inherent variability associated with use of a TLC, typically used for flow cytometrically-derived CD4 lymphocyte counts described at that time.

As disclosed by Melnicoff, it would have been important to the skilled artisan to use the gold standard of that time in order to adequately and accurately validate the new method described by the invention. Flow cytometrically-derived CD4+ lymphocyte counting would at that time have been the obvious standard to use, and use of true "total leukocytes" would have been contrary to the recommended guideline, especially if used as a gold standard from which to compare their invention. Melnicoff cite their own publication in this regard, which refers to use of TLC as the reference population for dual platform CD4+ lymphocyte enumeration

(*see* page 1, "Other Publications", *i.e.,* Landay and Muirhead, *Clin Immunol. Immunopath.* 52: 48-60, 1989). This paper refers directly to, and pre-empted, the subsequent publication of the widely accepted CD4+ lymphocyte enumeration guidelines "H-42A, 1989. *National Committee for Clinical Laboratory Standards, (NCCLS).*- Clinical Application of Flow Cytometry: Immunophenotyping of Lymphocytes; approved guideline". This guideline specifically states that Absolute/ Total Lymphocyte Count (TLC) be used in the dual platform calculation and not total leukocyte count.

Furthermore, the flow cytometric-derived CD4+ lymphocyte counts are not the primary focus of Melnicoff. Melnicoff describes a "method for determining presence or quantity of subsets of a subpopulation of cells, ...by means of uniformly incorporating a detectable reporter substance then separating the selected subset ....via affinity separation and detecting the reporter substance", col. 1, ln 18-30). In other words, Melnicoff describes a method of using affinity separation and subsequent detection with enzymatically-derived fluorescence to derive an estimate of an absolute CD4+ lymphocyte count. The flow cytometric-derived CD4+ lymphocyte counting is used by Melnicoff as the "reference standard" from which the performance of their invention was both validated and standardised (hence the need for development of a standardised curve, Example 3 A) to derive an estimate of an absolute CD4+ lymphocyte count.

Dorland appears to be cited to show that a Coulter counter is a kind of hematology analyzer which can be used to count the number of formed elements, such as leukocytes, in a cubic millimeter of blood, and that a flow cytometer is such a hematology analyzer (*see* Office Action, pages 7-8). However, the citation of Dorland does not change the deficiencies of Melnicoff, and is merely cited under 35 U.S.C. § 102 as a supporting reference. Pursuant to M.P.E.P. § 2131.01, a 35 U.S.C. § 102 rejection over multiple references has been held to be proper when the extra references are cited to prove the primary reference contains an "enabled disclosure"; explain the meaning of a term used in the primary reference; or show that a characteristic not disclosed in the reference is inherent. Therefore, Dorland cannot remedy the deficiencies of Melnicoff. Melnicoff does not recite each element

of the present claims, and does not have an enabling disclosure for the presently claimed subject matter.

In light of the above, Applicants request that the rejection under 35 U.S.C. § 102 be withdrawn.

Rejections Under 35 U.S.C. §103

Claims 2 and 5 stand rejected under 35 U.S.C. § 103 as purportedly obvious inlight of Melnicoff in view of Brando. Applicants traverse.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *See* MPEP § 2143.

Melnicoff, combined with Brando, fails to recite each element of the present invention, and to provide an expectation of success in practicing the present invention. As discussed above, Melnicoff does not disclose the enumeration of leukocytes, but rather discloses and leads the skilled artisan to the enumeration of total lymphocytes. Applicants submit that it would have been clear to the skilled artisan at the time Melnicoff was filed, that "leukocyte count" refers to the use of a total lymphocyte count. From review of the context in which "leukocyte count" is used in Melnicoff, it is clear that the error was not due to due to the belief that the total leukocyte count was being identified. A review of Melnicoff makes it clear that lymphocyte count was the intended purpose of the methods disclosed therein, as detailed above. Further, Melnicoff encourages the skilled artisan to use the best methods of the time, and the enumeration of leukocytes would have been contrary to this. Instead, flow cytometrically-derived CD4+ lymphocyte counting would at that time have been the standard to use.

Recommended guidelines for CD4+ lymphocyte enumeration by various international bodies have, consistently to date, recommended the use of total lymphocytes as the reference point for enumeration of CD4+ expressing lymphocytes, for both dual and single platform enumeration. Use of lymphocyte

reference, more recently as CD45+*bright cells,* continues to be recommended in the most recently published international guidelines (*See* Mandy et al. *MMWR Recomm Rep.* 2003 Jan 31; 52(RR-2): 1-13; Schnizlein-Bick et al., *Cytometry B Clin Cytom*, 2002, 50 (2); 46-52; Barnett et al., *Brit J Haem* 1999; 106: 1059-1062). Purity of the flow cytometric lymphoid gate was previously recommended with use of CD45+*bright* and CD14-> than 95% of the 'light scatter' lymphoid gate in the typical dual platform system prior to use of the leukocyte subset of brightly expressing CD45+ cells to define lymphocytes.

The latest CD4+ lymphocyte enumeration guidelines *continue* to recommend use of total lymphocytes and focus on use of single platform methodology to improve reproducibility and accuracy of CD4+ lymphocyte counting, citing poor reproducibility of haematology analyzer-derived total lymphocyte count (Centers for Disease Control (USA) "1997 Revised guidelines for performing CD4+ T-cell determinations in persons infected with human immunodeficiency virus (HIV)". *Morbidity and Mortality Weekly Report (MMWR)* 1997; 46:1-29*)*. The latest guidelines remain unchanged in their focus on identification of lymphocytes (*See* Mandy et al. *MMWR Recomm Rep.* 2003 Jan 31; 52(RR-2): 1-13; Schnizlein-Bick et al., *Cytometry B Clin Cytom*, 2002, 50 (2); 46-52; Barnett et al., *Brit J Haem* 1999; 106: 1059-1062.*)* as the primary step in enumeration of CD4+ lymphocytes. The NIH and the CDC also continue to recommend use of total lymphocytes, and not leukocytes, as the primary point of reference for enumeration of CD4+ lymphocytes.

Brando, combined with Melnicoff, fails to remedy the deficiencies of Melnicoff. Brando fails to disclose or even suggest the direct use of a white cell count to derive an absolute CD4+ lymphocyte count. Instead, Brando discloses the use of a three- or five-part leukocyte differential count and total lymphocyte count (*see* 329, col. 2) in the context of dual platform CD4+ lymphocyte enumeration. The use of a leukocyte count mentioned in relation to dual platform cell enumeration in Brando refers directly to dual platform CD34 stem cell enumeration, separate and different from CD4+ lymphocyte enumeration. Thus, Applicants submit that the combination of these two references fails to provide motivation to the skilled artisan to perform the method of the presently invention, or would suggest that a person of ordinary skill in the art would have anticipated success in performing the method.

Claims 1, 2, and 5 stand rejected under 35 U.S.C. § 103 as purportedly obvious in the light of Brando in view of Barnett. Applicants submit that the combination of Brando and Barnett fail to recite each element of the present invention or provide an expectation of success. Brando fails to disclose or even suggest the direct use of a white cell count to derive an absolute CD4+ lymphocyte count. Instead, Brando is directed to use of a three- or five-part leukocyte differential count and total lymphocyte count in the context of dual platform CD4+ lymphocyte enumeration. Barnett fails to remedy these deficiencies.

Further, Applicants respectfully submit that unexpected results are in fact present with respect to the claimed methods.

It is a well established legal precedent that the presence of an unexpected, advantageous or superior result is evidence of nonobviousness. *See* M.P.E.P. § 716.02(a); *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (C.C.P.A. 1963). Along these lines, it is also well established that "a greater than expected result" is evidence of nonobviousness. *See* M.P.E.P. § 716.02(a); *In re Corkill*, 711 F.2d 1496, 226 U.S.P.Q. 1005 (Fed. Cir. 1985).

Applicants provide the following information to further highlight that the use of total leukocytes in enumerating CD4+ lymphocytes was not obvious to a person of ordinary skill in the art at the time that the present patent application was filed, and was in fact, unexpected. Prior to the present invention, there had been no publication of a method of enumerating CD4+ lymphocytes with reference to the total leukocytes. The gating method described in claim 1 represents an improvement in the state of the art for both traditional dual and single platform methodologies. The strategy applied in the invention differs considerably from the state of the art, which typically requires use of both CD45+*bright* or light scatter identification of lymphoid cells in the first instance, with CD3 used in the second instance to define CD4+ T-cells (*see* Exhibit 1, attached). This approach is typically referred to as "dual anchor", and uses two anchoring gates.

In contrast, the present invention typically uses only total CD45++/ total leukocyte expression. The latter is easier to use, is not affected with sample age and has been shown to be a more reliable and reproducible method (both intra- and inter laboratory), for both dual and single platform methods. One significant benefit

of the present invention is in improving reproducibility in CD4+ lymphocyte enumeration, for both dual and single platform systems.

The ability to use a dual platform in the present invention represents a significant improvement of reproducibility over traditional dual platform CD4 lymphocyte counting. Specifically, many groups have advised use of a single platform methodology to avoid poor reproducibility associated with use of the poorly reproducible total lymphocyte count (TLC) in traditional dual platform systems (*See* Mandy et al. *MMWR Recomm Rep.* 2003 Jan 31; 52(RR-2): 1-13; Schnizlein-Bick et al., *Cytometry B Clin Cytom*, 2002, 50 (2); 46-52; Barnett et al., *Brit J Haem* 1999; 106: 1059-1062; and Mandy et al., *Cytometry (Communications in Clinical Cytometry)* 1997; 30: 157-165) and avoid use of TLC-referenced dual platform CD4+ lymphocyte counting. Dual platform PanLeucogating (*i.e.,* the method of the present invention), which uses a white blood cell count as opposed to a TLC, has significantly improved the reproducibility of traditional dual platform testing to bring it in line with, or even better than, the reproducibility of currently advocated single platform (beads) methods (*see* unpublished paper by Glencross et al., attached).

Although it appears to be the opinion of the Office that the use of beads would have been obvious to one of ordinary skill, the use of the simple gating strategy of the claimed invention has also been shown to improve the reproducibility of single platform testing. Therefore, it represents an improvement on existing single platform methodologies. If the simple gating strategy applied in the present invention is used in the context of single platform (bead) counting instead of the state of the art gating described in various guidelines, this methodology can further improve the reproducibility between laboratories that currently use single platform methodology.

In the attached unpublished paper by Glencross, evidence is provided in support of the above and demonstrates the improvement with PLG use over traditional dual and single platform methods (which rely on use of the total lymphocyte count or CD45bright expression to identify lymphocytes as the point of reference for enumeration of the CD4+ lymphocytes. This data has been further confirmed in a 5-laboratory site validation study performed under the auspices of the US NIH to evaluate the "new" Panleucogating strategy versus the state of the art "predicate" gating (to be published shortly). In support of the argument for

PanLeucogating (the method of he invention), the NIH initiated and supported a validation study to evaluate the PanLeucogating strategy versus the current "predicate" dual and single platform methods based on traditional reference/ method using total lymphocytes.

Therefore, although the method of the present invention affords significant advantages that it may seem obvious with hindsight, it is submitted that this was not the case at the time the patent application was filed. Evidence of the controversy and criticism surrounding the concept of CD4 enumeration has also been documented in the medical literature [*see S. Afr. Med. J.* 2002 Aug; 92(8):5723, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=12244607&dopt=Abstract].

Finally, Applicants note that the inventor has received much recognition for the present invention as a result of her work. D.K. Glencross was invited in 2005 to present a plenary talk at the 5th European Working Group for Cell Analysis meeting, held in Athens from 21-23 September, to present the data on the concept and use of white cell count single anchor gating for CD4+ lymphocyte enumeration (*see* http://www.cytometry-athens2005.org/programme.htm). In addition, she has received recognition for this work, including a South African National Science Technology Forum (NSTF) Award, for Research and Innovation over previous two years (a peer-review award from the South African Government National Department of Science, Engineering and Technology of South Africa for Outstanding Contribution to the Science in South Africa). She also received the South African NATIONAL PRODUCTIVITY INSTITUTE Gold Award in 2003. This award is given based on the following criteria: that the productivity has improved through innovation; that the improvement steps have been clearly recorded and that it is demonstrated to be sustainable over at least 18 months; that the benefits are clearly outlined and that the initiative has resulted in a competitive advantage; and that a culture focusing on productivity and ongoing improvement has been developed by the individual awarded. (Please see http://www.npi.co.za/productivitypage/Prod.htm). Dr. Glencross has also received the U.S. based International JPMorgan-Chase Health Award Laureate, in 2002, awarded for Technology Innovation in Health. In 2002, her

work was recognized by the World Health Organization. Thus, her work as claimed in the present application is unexpected, and has provided great benefits to the art.

In light of the above, Applicants request that the rejections under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

It is respectfully submitted that all rejections have been overcome by the above amendments. Thus, Notice of Allowance is respectfully requested.

In the event that there are any questions relating to this Amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (703) 836-6620 so that prosecution of the application may be expedited.

Respectfully submitted,

BUCHANAN INGERSOLL PC
(INCLUDING THE ATTORNEYS FROM BURNS DOANE SWECKER & MATHIS)

Date: December 12, 2005

By: _____
Deborah H. Yellin
Registration No. 45,904

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Enclosures: Exhibit 1
Copy of unpublished paper by Glencross et al. entitled "Pan*leuco*gated (Plg) Cd4 Counting: A Cost Effective, Simple And Reproducible Solution For Hiv/ Aids Monitoring In A Resource Limited Setting"
Copy of *MMWR Recomm Rep*. 2003 Jan 31; 52(RR-2): 1-13; Schnizlein-Bick et al., *Cytometry B Clin Cytom*, 2002, 50 (2); 46-52; Barnett et al., *Brit J Haem* 1999; 106: 1059-1062, by Mandy et al.
Copy of page 1, "Other Publications", *i.e.,* Landay and Muirhead, *Clin Immunol. Immunopath*. 52: 48-60, 1989
Copy of Debate Athens http://www.cytometry-athens2005.org/programme.html
Copy of Multi-platform equivalency of PLG CD4 by Glencross et al.